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Artigos

Internal and external morphology of *Tectona grandis* Linn F. diaspores and its relation to seed quality

Morfologia interna e externa de diásporos de *Tectona grandis* L. e sua relação com a qualidade de sementes

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ABSTRACT

Bearing in mind the importance of *Tectona grandis* L. production in Mato Grosso State, Brazil, it is necessary to establish crops with seedlings of excellent quality, and the selection of diaspores can improve seed vigor, thus providing better quality to the seedlings. It was aimed to indicate the ideal stage of maturation of *T. grandis* diaspores through image analysis, guaranteeing seed quality and seedling production. *T. grandis* diaspores were collected in the years 2016 and 2017 at 90, 120, 150, and 180 days after anthesis. The material collected was analyzed for the presence of seeds per fruit in X-ray equipment to compose the treatments. After this selection, the variables color, area, and diameter were analyzed, and fruit weight, germination, and emergence tests were carried out. The diaspores along the maturation process, had higher amount of seeds and higher weight. The increase in the colors yellow and black was na indicative of the maturation for *T. grandis* diaspores collected in 2017, and the reduction of the percentagem of dark gray color for the diaspores collected in 2016. Fruit area and diameter was higher in those who had four seeds and the diaspore quality evaluated by the germination percentage, germination first count, initial stand and emergence percentgevary according to the amount of seeds present in the diaspore, being higher for diaspores containing 4 seeds. Diaspore quality was evaluated by germination percentage, first germination count, initial stand, and emergence percentge, was superior in the 2017, with diaspores having 2 na 3 seeds. The maturation of *T. grandis* diaspores is dependent of the year of collection and presents better quality when collected at 120 and 150 days after anthesis.

Keywords: Seed image analysis; Maturation; Breaking seed dormancy

RESUMO

Tendo em vista a importância da produção de *Tectona grandis* Linn F. no estado de Mato Grosso, é necessário estabelecer cultivos com mudas de excelente qualidade e a seleção de diásporos pode favorecer seu vigor, proporcionando a produção de mudas de maior padrão. O objetivo da pesquisa foi indicar o estágio de maturação ideal dos diásporos de teca por meio da análise de imagens, que garanta a qualidade de sementes e produção de mudas. Diásporos foram coletados nos anos de 2016 e 2017 aos 90, 120, 150 e 180 dias após a antese e analisados quanto à presença de sementes por fruto em equipamento de raios X para compor os tratamentos. Após a seleção, foram analisadas características de cor, área e diâmetro e realizados testes de peso de frutos, germinação e emergência. Os diásporos durante o processo de maturação possuíam maior peso e quantidade de sementes. O aumento da porcentagem da cor amarela e preta foi indicativo da maturação dos diásporos coletados em 2017 e a redução da porcentagem da cor cinza escuro para o ano de 2016. A área e diâmetro dos frutos foram maiores naqueles que continham quatro sementes e a qualidade dos diásporos avaliados, pela germinação, primeira contagem de germinação, estande inicial e emergência, foi superior no ano de 2017, com diásporos contendo 2 e 3 sementes. A maturação dos diásporos de teca são dependentes do ano de coleta e com melhor qualidade quando coletados aos 120 e 150 dias após a antese.

Palavras-chave: Análise de imagens de sementes; Maturação; Superação de dormência de sementes

1 INTRODUCTION

Native to the Asian continent, *Tectona grandis* Linn. F. or teak (popular name) has adapted well to the edaphoclimatic conditions of Mato Grosso State, Brazil. It is one of the main species cultivated for wood production, with use in several sectors of the industry (IBA, 2019). This adaptation is particularly noticeable in terms of both the planted area in the state - with 93,957 ha (IBA, 2019) - and the wood cutting period. In Mato Grosso State, this species can be cut after 25 years of implementation, corresponding to a reduction of 55 years in relation to the cutting period in its region of origin (GIUSTINA; ROSSI; VIEIRA; TARDIN; NEVES; PEREIRA, 2017). The Brazilian production, mainly from Mato Grosso State, has the Asian market as the main destination.

As one of the most valued tree species on the market, teak is known worldwide for its wood strength and durability (SOUZA; GONÇALEZ; RIBEIRO; BAHIA, 2019). It stands out in the production of furniture, frames, floors, shipbuilding, panels, veneers, and wainscoting (COIMBRA; VAZQUEZ; NOGUEIRA, 2014).

As for the implantation of silvicultural areas of this species, seedlings can be produced either by seeds or by means of asexual propagation. The use of seeds for this purpose requires the consideration of several factors for the formation of quality seedlings. These include the ideal collection period, the choice of parent plants, environmental conditions, and the overcoming of diaspore dormancy.

In this context, the diaspore maturation stage can indicate the physiological maturity of teak seeds, which is the main parameter used by seed producers of this species. Notwithstanding, the hardness of these diaspores - which are quadrilocular and can contain from 0 to 4 seeds - and the difficulty of extracting seeds prevent the evaluation of morphological structures of seeds capable of originating a normal seedling. In this way, image analysis techniques to assess the internal morphology of seeds can be a tool to establish the ideal moment for fruit collection. This period coincides with the formation of the essential structures of the seed, thus guaranteeing the production of higher quality seedlings.

The evaluation of the internal morphology of seeds and fruits has been supported by image analysis techniques (RAHMAN, CHO, 2016; HUGHES; ASKEW; SCOTSON; WILLIAMS; SAUZE; CORKE; DOONAN; NIBAU,2017), mainly through X-ray. This tool provides a quick and efficient evaluation of the internal structures of seeds in most agricultural and forestry species (FARIA; PAIVA SOBRINHO; OLIVEIRA; TAVARES; LUZ, 2021; JEROMINI; MARTINS; PEREIRA; GOMES–JUNIOR, 2019; MENEZES; CÍCERO; VILLELA; BORTOLOTTO; 2012; ABUD; CICERO; GOMES JUNIOR; 2018; MARCHI; GOMES-JUNIOR, 2017; NORONHA; MEDEIROS; PEREIRA, 2018; MEDEIROS ARAUJO; LEÓN; SILVA; DIAS, 2018; PINHEIRO; MEDEIROS; SOARES; CAPOBIANGO; DIAS, 2022). In addition to being a nondestructive technique, the evaluated seeds can be submitted to other tests necessary to prove seed quality. This technique is widely used in the selection of seeds for the composition of germplasm banks, enabling the storage of seeds with greater viability (LIU; BREMAN; COSSU; KENNEY, 2018). The technique provides information on morphological abnormalities or possible damage that may impair germination (NORONHA; MEDEIROS; PEREIRA, 2018).

Another technique that can be used to evaluate seed morphology is computerized analysis. From the capture of seed images, this technique categorizes characteristics aggregated to size, color, shape, texture, among others, which are acquired quickly (FARIA; PAIVA SOBRINHO; OLIVEIRA; TAVARES; LUZ, 2021). The field of vision with the capture of images, associated with classification techniques, provides an adequate overview for automatic identification of seeds (ANDRADE, OLIVEIRA, PINTO, PIRES, OLIVEIRA, SILVA, CARVALHO, 2016; MARCHI; GOMES JUNIOR, 2017; MARQUES; ASSIS; BUSTAMANTE; ANDRADE; CARVALHO; LOPES, 2019). However, studies related to computerized analysis of seeds are recent, requiring more research related mainly to the association of evaluation methodologies of morphological characteristics with seed quality.

In view of this information, the present research uses image analysis to evaluate teak diaspores collected at different stages of maturation, verifying its effects on seed quality and seedling production.

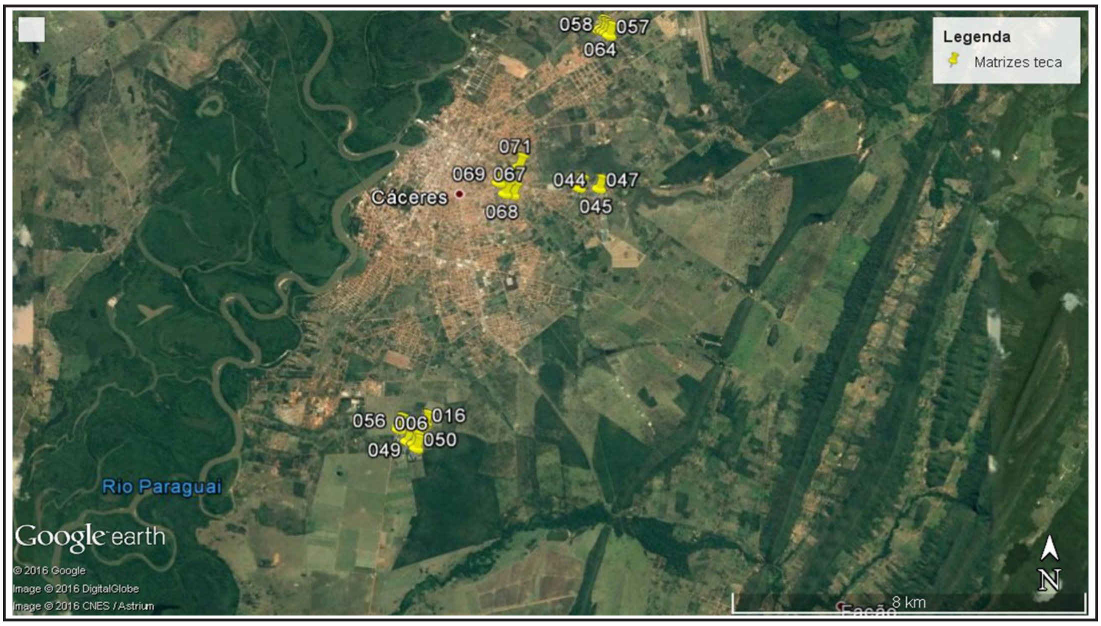
2 MATERIALS AND METHODS

The research was carried out at the Laboratory of Plant Science of the University of Mato Grosso State, Cáceres Campus, and at the Central Laboratory of Seeds, Department of Agriculture, School of Agricultural Sciences of Lavras, Federal University of Lavras (UFLA), in Lavras - MG. In order to meet the objectives of the study, diaspores were collected from populations of the species *Tectona grandis* located in Cáceres city. Fifteen matrices were selected and mapped using GPS (Figure 1) considering a minimum distance of 100 meters between trees, a height of 5 m, and the absence of pests and diseases.

Samples were collected between March and June 2016 and 2017 at 90, 120, 150, and 180 days after anthesis. Climatological data collected from the local main station were computed in the same period. After collection, a bulk of these diaspores was formed, and water content was initially determined according to the guidelines of the

Rules for Seed Analysis (BRAZIL, 2009). After the determination, moisture was adjusted to 9% by drying in a forced air circulation oven. After adjusting fruit moisture content, the exocarp was removed by rubbing in a sieve.

Figure 1 – Marking of the teak matrices used to collect diaspores during the years 2016 and 2017

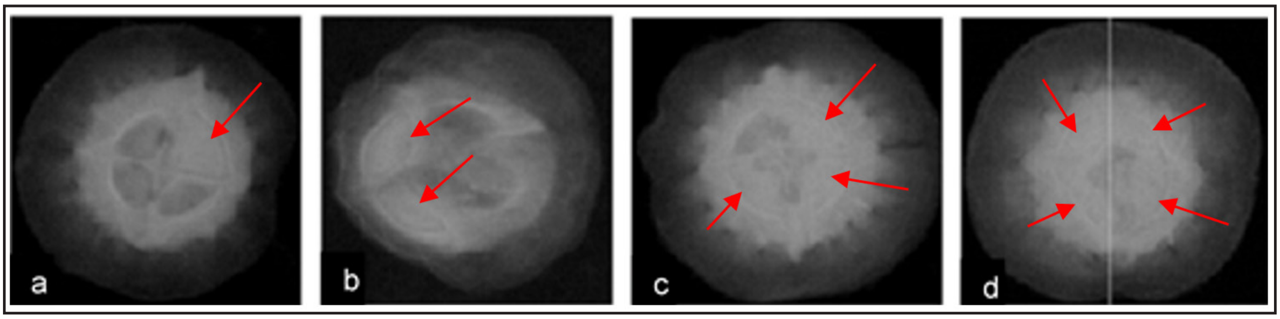


Source: Authors, adapted from Google Earth (2018)

For the analysis of internal morphology, diaspores were arranged on Styrofoam plates and placed in Faxitron MX-20 DC 12 equipment with automatic adjustment of exposure time and radiation intensity to obtain the radiographic images. After these images were obtained, each diaspore was classified according to the number of seeds, ranging from one to four seeds per fruit (Figure 2).

The external morphology of seeds was analyzed by the Groundeye® S800 Image Analysis System version 1.3.5, consisting of a capture module and a software to analyze the captured images. The seeds of each treatment were placed in the tray of the equipment and the images were captured by high resolution cameras. The following characteristics were analyzed: color dominance; maximum, minimum, and Feret diameter of diaspores.

Figure 2 – Radiographic images of teak diaspores containing 1 to 4 seeds. (a) 1 seed; (b) 2 seeds; (c) 3 seeds; (d) 4 seeds



Source: Authors (2022)

After obtaining the images and classifying the diaspores according to the amount of seeds present, diaspore weight was determined according to the methodology proposed by Brazil (2009). For the germination and emergence tests, 100 diaspores per treatment were submitted to dormancy breaking with immersion in water for 48 hours (CASTILHO; FARSONI; ROSSI, 2014). For the germination test, the seeds (subdivided into repetitions of 25) were sown in plastic trays containing vermiculite substrate and moistened to 60% of the water holding capacity. A photoperiod of 12 hours was used along with a temperature between 25-35 °C (CASTILHO; FARSONI; ROSSI, 2014). Counts were performed at 14 (first count) and 28 days according to the Rules for Seed Analysis (BRAZIL, 2009), and the results expressed in percentage.

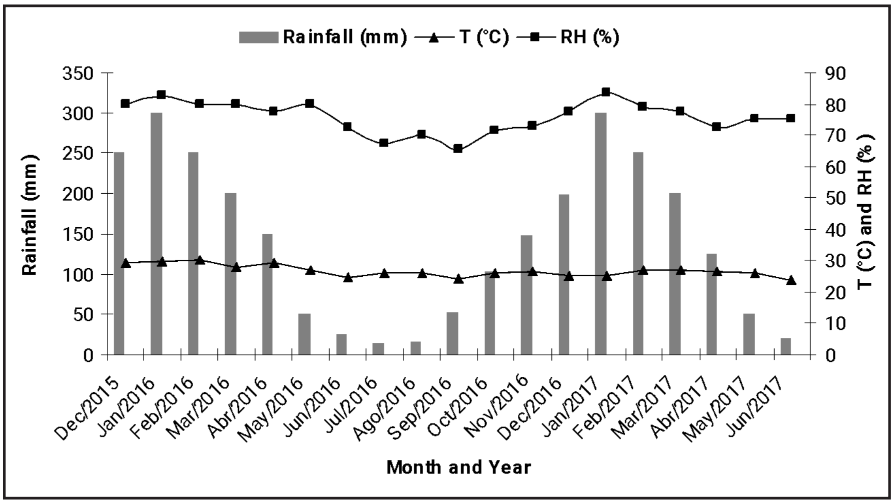
For seedling emergence assessment, 100 diaspores subdivided into four replications were used per treatment. The diaspores were sown in 110 cm^3 tubes with Plantmax® substrate under a 50% shade screen. Evaluations covered the initial stand at 30 days and the emergence stand after 90 days of test. The results were expressed in percentage of emerged seedlings.

The experimental design was completely randomized in a 4x2x4 split-plot scheme corresponding to four stages of diaspore collection (90, 120, 150, and 180 days after anthesis), two years of collection (2016 and 2017), and the classification of the number of seeds present in the diaspore (1, 2, 3, or 4 seeds). Response variables were submitted to analysis of variance. Subsequently, graphs were prepared with response surfaces to analyze the behavior of the variables under study.

3 RESULTS AND DISCUSSIONS

Teak anthesis occurs in December in Cáceres - MT, coinciding with the wettest period for the center-west region (Figure 3). For each year, diaspore collections started in March and ended in June. Rainfall levels decrease in this period while relative humidity and average temperature remain stable. The two years of collection showed the same pattern of values for temperature, relative humidity, and rainfall.

Figure 3 – Temperature (T °C), Relative Humidity (RH %), and Rainfall (mm) during the anthesis and collection period of teak diaspores



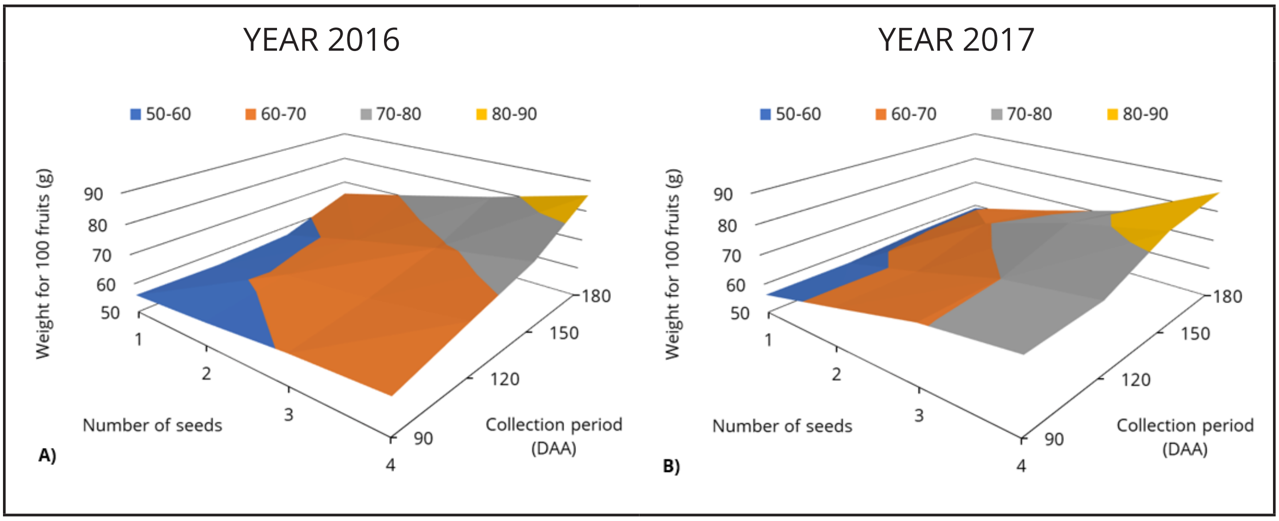
Source: Authors (2022)

With the advance of maturation and the increase in number of seeds, the weight of 100 fruits increased, being more pronounced in fruits collected in 2017 (Figure 4B) than in those collected in 2016 (Figure 4A).

Fruit mass accumulation occurs in several species as they mature, possibly indicating physiological maturity of seeds, coinciding with greater germination and vigor (DORNELAS; ALMEIDA; FIGUEIREDO NETO; SOUSA; EVANGELISTA, 2015; SCHULZ; SCHNEIDER; GUSATTO; IGNÁCIO; MALAVASI, 2017).

With the maturing of diaspores from anthesis, loss of water occurs naturally. In each collection period, moisture was 52%, 35%, 26%, and 13%, reaching a percentage of up to 9% through natural drying, when the tests started. As teak is a tetralocular, drupe fruit (ANGELI and STAPE, 2013), water loss occurs more slowly, requiring longer drying periods until reaching the desired moisture for the tests. Drying time was up to 30 days.

Figure 4 – Weight of 100 fruits according to the number of seeds and collection period (days after anthesis - DAA) in two years (2016 - A and 2017 - B)



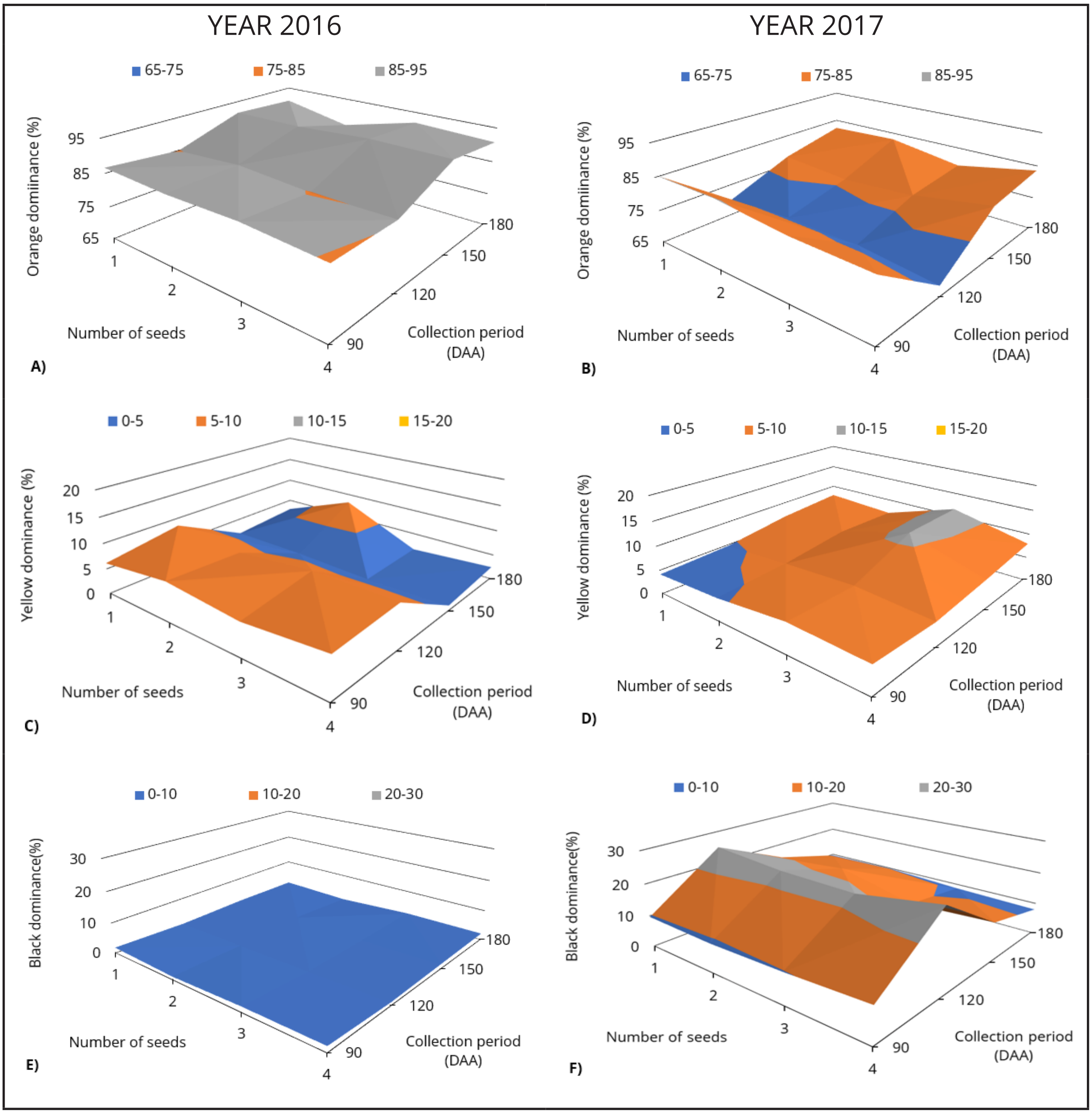
Source: Authors (2022)

In addition to water loss, maturation involves changes in the color of diaspores, with a predominance of orange, yellow, and black colors. This variable depends on maturation stage and year of production. These differences can be evidenced when evaluating the season, the year of collection, and the amount of seeds per diaspore (Figure 5).

Orange predominated among the colors identified through the RGB pattern, although with different responses for each year evaluated. The dominance of this color increased with the maturation of diaspores in 2016 (Figure 5A). On the other hand, in 2017 (Figure 5B), the dominance of orange color changed during the periods under study.

The dominance of the yellow color decreased significantly from 120 to 150 DAA in 2016 (Figure 5C). In 2017 (Figure 5D), this dominance increased slightly as the number of seeds per diaspore increased and with the advance of maturation. The only exception was for diaspores with two seeds, which showed a significant increase in the dominance of yellow color for the same collection period observed in 2016.

Figure 5 – Orange (A and B), yellow (C and D), and black (E and F) dominance colors in teak diaspores according to the number of seeds and collection period (days after anthesis - DAA) in two years (2016 and 2017, respectively)



Source: Authors (2022)

Although not very expressive, black was the color that most differentiated in the two years. In 2016 (Figure 5E) its values remained stable throughout the collection period, regardless of the number of seeds per diaspore. In 2017 (Figure 5F) the dominance of black color increased from 90 to 120 DAA, followed by a reduction until

180 DAA. One can thus infer that the pattern of dominance of black color correlates with the maturation of diaspores.

Color is a visual characteristic that can indicate the fruit and seed maturation process. Studies on *Comanthera* sp. to verify changes in inflorescence color during maturation by means of computerized image analysis made it possible to distinguish colors. The results highlighted the dominance of orange and black colors (MARQUES; ASSIS; BUSTAMANTE; ANDRADE; CARVALHO; LOPES, 2019). This behavior corroborates the results of the present study, in which the dominance of orange and black colors indicates variations during diaspore maturation.

The amount of seeds correlates with the area per diaspore, which can be a useful tool for the selection of materials for the formation of teak seedlings. However, this research showed that the year of collection influences diaspore size, with overall larger dimensions (area and diameters) in 2017 than in 2016 (Table 1).

Table 1 – Absolute frequency of the area (cm^2), mean Feret diameter, and maximum and minimum diameter of teak diaspores in two years of collection

Class	Area (cm^2)		Diameter (cm)					
			Feret		Maximum		Minimum	
	2016	2017	2016	2017	2016	2017	2016	2017
1.20 – 1.40	0	0	0	0	0	0	9	0
1.41 – 1.60	0	0	11	0	12	0	6	13
1.61 – 1.80	9	1	5	13	4	12	0	3
1.81 – 2.00	4	3	0	3	0	3	0	0
2.10 – 2.20	2	9	0	0	0	0	0	0
2.21 – 2.40	1	3	0	0	0	0	0	0

Source: Authors (2022)

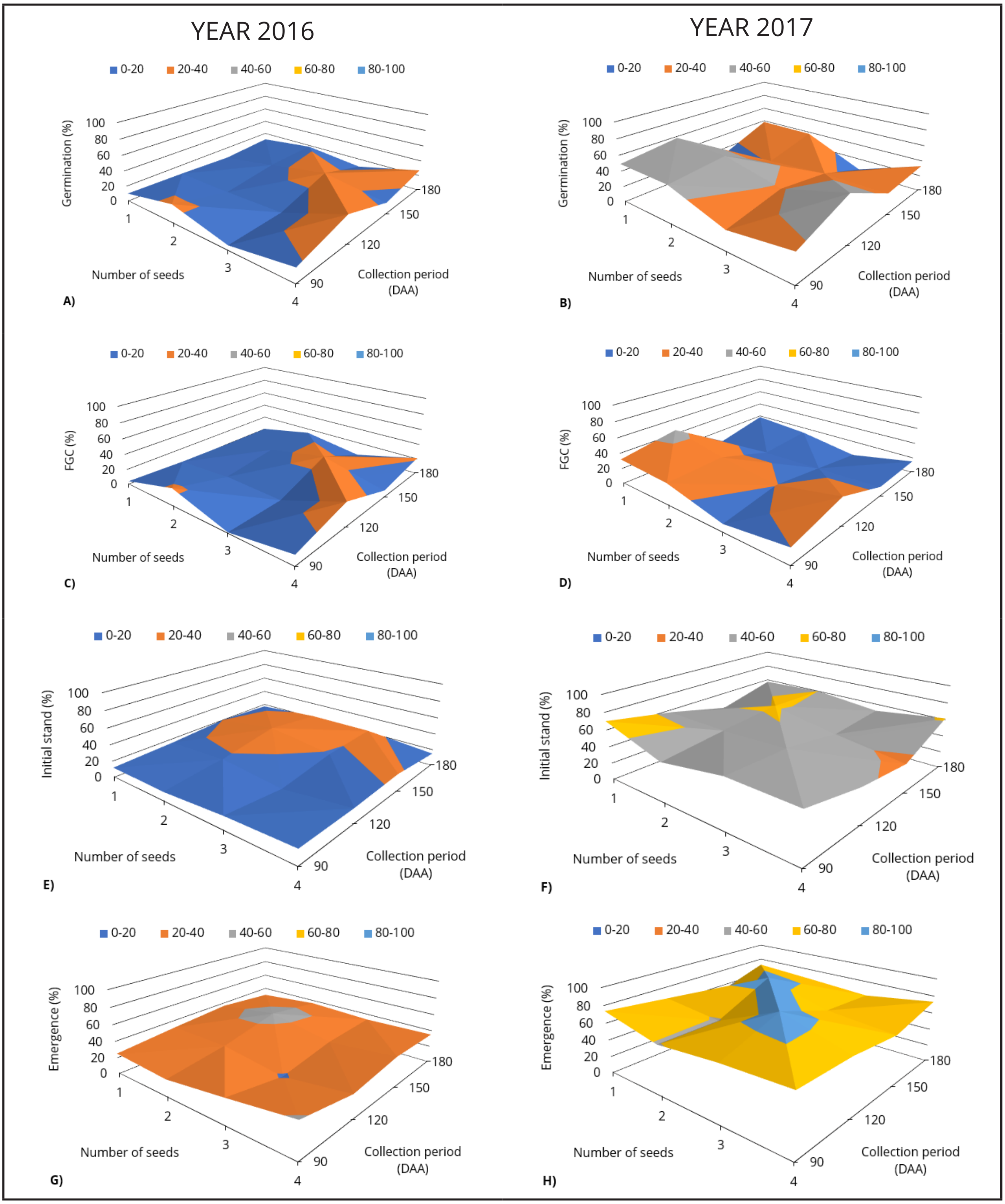
In the collection carried out in 2016, diaspore area ranged from 1.61 to 2.40 cm^2. In 2017, the area of most diaspores remained between 2.10 and 2.20 cm^2. Diameter behaved similarly to diaspore area, with small differences according to the year of collection.

Seed size is a physical characteristic determined by genotype and influenced by environmental conditions that occur during seed formation. Regarding this characteristic, it is remarkable how the year of collection greatly influences seed diameter, including the maximum, minimum, and Feret diameter. Studies recommend using these characteristics for measuring plant materials through image analysis as they allow for more efficient differentiation (MARQUES; ASSIS; BUSTAMANTE; ANDRADE; CARVALHO; LOPES, 2019). In the case of plant structures such as diaspores, which do not have a defined geometric shape, Feret diameter can be more accurate than other measures that apply to structures of more regular shapes (GARGIULO; GRIMBERG; REPO-CARRASCO-VALENCIA; CARLSSON; MELE, 2019).Both the external morphology and the maturation of diaspores directly interfere with seed quality, with differences depending on the year of collection. With regard to germination percentage, diaspores collected in 2016 had low viability (Figure 6A) in relation to those collected in 2017 (Figure 6B).

In 2017, for the collection carried out at 90 DAA, diaspores containing one and two seeds had a higher germination percentage than those containing three and four seeds. In turn, for the collection performed at 120 DAA, the values for this variable were higher regardless of the number of seeds (Figure 6B). The first germination count, which is a variable that analyzes vigor, showed similar behavior (Figure 6D).

Germination was not higher than 60% even when diaspores were submitted to dormancy breaking with immersion in water for 48 hours, as proposed by Castilho; Farsoni; Rossi (2014). Reports in the literature associate low teak germination with other types of dormancy, which can be physical or chemical, and with embryo immaturity (FERREIRA; CAMARGO; SOUZA JÚNIOR; SOUZA; OLIVEIRA, 2016; GEORGIN; BAZZOTI; PERRANDO, 2014; SLATOR; CALLISTER; NICHOLS, 2013). The combination of these factors, even when associated with dormancy overcoming methodologies, can still result in a low germination percentage.

Figure 6 - Germination percentage (A and B), first germination count (FGC - % - C and D), initial stand (% - E and F), and percentage of emergence (G and H) of teak diaspores according to the number of seeds and collection period (days after anthesis - DAA) in two years (2016 and 2017, respectively)



Source: Authors (2022)

4 CONCLUSIONS

The maturation of teak diaspores is dependent on the year of collection and with better quality when collected at 120 and 150 days after anthesis.

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